Magnetic Resonance Imaging Study on the Effect of Levemopamil on the Size of Intracerebral Hemorrhage in Rats

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Background and Purpose Beneficial effects of calcium antagonists in cerebral ischemia and trauma have been attributed in part to improved cerebral blood flow. Enhancement of cerebral blood flow, however, could aggravate the pathological situation if brain injury is associated with intracerebral hemorrhage. In this study we used high-field magnetic resonance imaging in an animal model of intracerebral hemorrhage to determine noninvasively the effect of the calcium and serotonin antagonist levemopamil [international nonproprietary name for (S)-emopamil] when infused in a dose (6 mg/kg) that is known to increase cerebral blood flow.

Methods Intracerebral hemorrhage was induced in rats by stereotaxic microinfusion of collagenase into the caudate putamen. Two series of experiments were performed. (1) Levemopamil was intravenously infused 30 minutes after intracerebral infusion of collagenase (0.05 U), which represents the time of intracranial bleeding. Another group of animals was given heparin (55 IU • kg⁻¹ • min⁻¹) to evaluate the capability of this animal model to demonstrate drug-induced worsening of intracerebral hemorrhage. (2) The effects of hyperacute infusion of levemopamil (30 minutes after infusion of 0.5 U of collagenase) were compared with those of a 2-hour delayed administration. In both experimental settings, the extent of intracerebral hemorrhage was determined by T₁-weighted magnetic resonance images (spin-echo; repetition time, 400 milliseconds; echo time, 23 milliseconds) taken in vivo in a coronal and a transverse brain plane 24 hours after collagenase infusion.

Results (1) Hemorrhagic brain areas measured 10.1±2.9 mm², 8.5±2.1 mm², and 18.8±2.5 mm² in the coronal brain plane (10 mm anterior to the interaural line) of control, levemopamil-, and heparin-infused rats, respectively (8 animals per group, mean±SD). In the transverse brain plane (6 mm dorsal to the interaural line) the hemorrhagic area was 11.5±3.6 mm², 9.7±2.4 mm², and 19.9±3.3 mm² in control, levemopamil-, and heparin-infused rats, respectively. (2) Animals with 2-hour delayed levemopamil infusion displayed intracerebral hemorrhage similar in size to that of control rats. (3) Neither small nor large hemorrhagic lesions were increased by levemopamil.

Conclusions Agragravation of intracerebral hemorrhage was not observed by magnetic resonance imaging in levemopamil-infused animals. However, infusion of heparin caused a significant (P<.05), almost twofold increase in the size of intracerebral hemorrhage. These results justify clinical trials with levemopamil in cerebral disorders such as stroke, brain trauma, and peritumoral brain edema, which may be accompanied by intracerebral hemorrhage from the beginning or where transition to intracerebral hemorrhage may occur.

See Editorial Comment, page 1841.

Key Words • calcium channel blockers • cerebral hemorrhage • heparin • magnetic resonance imaging • serotonin • rats
lead to increased bleeding and intensify the detrimental consequences of ICH. Thus, the aim of the present study was to measure the effect of levemopamil on the extent of ICH, when intravenously infused in a high dose that is known to increase CBF in rats. The experiments were performed in a rat model of intracerebral bleeding that has shown close histopathological correspondence to ICH in humans. In one part of this study the effects of levemopamil were compared with those of heparin, which was used to evaluate the capability of this animal model to demonstrate drug-induced augmentation of ICH. Infusion of heparin was chosen because heparin is widely used in the treatment of stroke patients, and x-ray computed tomography (CT) has been recommended to exclude ICH before anticoagulant therapy.

In the second part of this study two different time schedules of levemopamil administration were tested with respect to possible clinical situations. One group of animals received an infusion of levemopamil at the time of intracerebral bleeding (half an hour after collagenase infusion). This is considered the worst case, which could be equivalent to a very early start of levemopamil therapy in stroke patients, during transportation to the hospital. Another group of animals levemopamil infusion was begun after a delay of 2 hours, which should resemble early clinical arrival of patients presenting with ICH.

The extent of ICH was determined by magnetic resonance imaging (MRI) in vivo, since the quantification of blood after extravasation into tissue with degraded extracellular matrix may be more accurate when measured noninvasively in situ than after slicing and embedding of enzymatically destroyed brain tissue for conventional histology. Moreover, clinical MRI has become a useful tool in the detection of hemorrhagic lesions and their correlation with clinical neurological symptoms and signs.

Materials and Methods

Male Sprague-Dawley rats weighing 280 to 330 g were anesthetized with 13 mg/kg IP fluanisone and 0.4 mg/kg IP fentanylhydrogen citrate (Hynpomf, Janssen Neuss). Collagenase was infused into the caudate putamen as described by Rosenberg et al. Rats were placed on a feedback-controlled heating table, and a burr hole was drilled into the skull. The head was fixed in a stereotaxic apparatus (David Kopf Instruments). A vertical needle was introduced 5.5 mm into the right brain hemisphere 3.5 mm lateral to the midline and 10 mm anterior to the interaural line. A microinfusion pump (Injektomat, Fresenius) was used to infuse 2 μL of a saline solution of collagenase (type CLS, Biochrom Berlin) over 10 minutes. After infusion the needle was removed, and the wound was sutured. Animals recovered in a warm place with access to food and tap water. In the first series of experiments the infused amount of collagenase was 0.05 U, and groups of treatment were as follows: (1) control (0.9% NaCl solution); (2) 6 mg/kg levemopamil; and (3) 10 mg/kg unfractionated heparin sodium (166 IU/mg, Nordmark Uetersen). Drugs were dissolved in distilled water and infused via the tail vein beginning 30 minutes after the end of collagenase infusion. The drug infusions lasted 30 minutes, and the infused volume was 2 mL.

In a second series of experiments 0.5 U of collagenase were used, and 6 mg/kg levemopamil were infused via the tail vein either 30 minutes after (n=9) or 2 hours after (n=8) the end of collagenase infusion. Controls (n=8) were infused with 0.9% NaCl solution 2 hours after collagenase infusion. Again, the infusions of levemopamil or saline solutions lasted 30 minutes (volume, 2 mL). Sham-operated animals received an intracerebral infusion of 0.9% NaCl solution without collagenase. Animals were reanesthetized for MRI 24 hours after collagenase infusion.

MRI was carried out on a General Electric CSI-II 2.0-T nuclear magnetic resonance (NMR) spectrometer equipped with Acuscan self-shielded gradient coils (maximum gradient strength, ±20 G/cm; 15-cm inner bore diameter). High-field MRI (85.542 MHz) was performed with a home-bred low-pass birdcage proton-imaging coil, as has been described in detail elsewhere. Body temperature of the animals was maintained constant by a continuous flow of warm air into the bore of the magnet. The water signal of the head was shimmed to a proton line width of typically 0.6 ppm. T1-weighted spin-echo images (repetition time, 400 milliseconds; echo time, 23 milliseconds) were obtained with a field of view of 30 mm, in which 16 scans were averaged for each one of the 128 phase-encoding steps, resulting in a total acquisition time of 28 minutes. Images (slice thickness, 2 mm) were acquired in a transverse plane 6 mm dorsal to the interaural line and a coronal plane 10 mm anterior to the interaural line.

After the NMR data were processed with standard GE/MRI software on a Nicolet 1280 computer, the images were quantified by planimetry by means of a computerized image analysis system (Kontron CARDO 290) with respect to the total brain area and the hemorrhagic area, including the hypointense rim. All results are presented as mean±SD. Comparison was performed using ANOVA and Newman-Keuls multiple range test. Differences were considered significant at P<.05.

Results

Infusion of collagenase into the caudate putamen of rats caused a highly reproducible ICH in the right hemisphere. T1-weighted MR images taken 24 hours after collagenase infusion displayed the ICH in general as an isointense or slightly hyperintense region with a hypointense rim (Figs 1 and 2). The space-occupying lesions produced backward displacements of the lateral ventricle on the ipsilateral side and also brain midline shifts to the contralateral side. No such pathological changes were detected in sham-operated animals receiving an intracerebral infusion of physiological saline solution instead of the collagenase solution (Fig 3).
The effects of intravenous infusions of levemopamil and unfractionated heparin on the extent of ICH were investigated. The results of the quantitative analyses of coronal and transverse images are summarized in the Table. In heparin-infused animals ICH was significantly increased to almost twice the size of that in control animals. In contrast, a slight decline (16%, \( P=\text{NS} \)) of the hemorrhagic area in both the coronal and transverse brain planes emerged under levemopamil treatment.

Calculation of the relative hemorrhagic areas, taking into account possible differences in total brain areas of individual rats, yielded results that showed good correspondence with the absolute values for both drugs (Figs 4 and 5). Drug-induced changes in the absolute and relative hemorrhagic areas were of the same degree in coronal and transverse brain planes.

In the second part of this study higher amounts of collagenase than in the first part were infused (0.5 U versus 0.05 U), and the effects of early (30 minutes after collagenase infusion) and delayed (2 hours after collagenase infusion) levemopamil infusions (6 mg/kg IV, 30 minutes) were investigated. As could be expected from the literature, the size of the hemorrhagic lesion was increased when higher amounts of collagenase were infused. Thus, the relative hemorrhagic area was increased from 11.7±3.0% and 4.9±1.4% in the coronal and transverse brain planes, respectively, of control animals in the first part of this study (Figs 4 and 5) to 25.3±2.8% and 13.4±3.4% in coronal and transverse planes, respectively, of the controls in the second part of the study (Fig 6).

Under these experimental conditions, again no increase of hemorrhagic areas was observed in both brain planes of levemopamil-treated animals, suggesting (1) that neither small nor large ICH is increased by levemopamil and (2) that neither hyperacute nor 2-hour delayed levemopamil treatment leads to an aggravation of the hemorrhagic lesion.

### Discussion

In the present MRI study an aggravation of intracranial bleeding by intravenous infusion of levemopamil was not observed despite administration of a dose that has been shown to increase CBF in the rat. The experiments were performed in a rat model as described by Rosenberg et al by infusing collagenase into the caudate putamen to induce intracranial bleeding. Collagenase is a proteolytic enzyme that occurs in an inactive form in cells. It is released and activated during injury, leading to disruption of the extracellular matrix and extravasation of blood cells. Caudate and putamen are common sites of bleeding in 40% of patients with ICH. Thus, this animal model mimics pathological processes as they may take place in stroke.
patients. Since large cerebral blood vessels are also affected, it could be expected that levemopamil might enhance bleeding when administered in a CBF-increasing dose.

The use of varying experimental approaches revealed that neither small nor large ICH is increased by levemopamil. Furthermore, no enlargement of ICH has been found after levemopamil infusions that have been started at different times after collagenase infusion in relation to possible clinical situations. In one experimental group the infusion of levemopamil was begun after 30 minutes. This is the time of bleeding when intact erythrocytes are dissecting between normal brain cells. Similarly, levemopamil could be administered by chance to a patient in a very early stage of hemorrhagic stroke. Another group of animals received levemopamil 2 hours after collagenase infusion, corresponding to early patient admission to the hospital. Taken together, our results show that levemopamil does not provoke adverse effects on ICH independent of the size of ICH and the time of treatment. Thus, CT may not be mandatory to rule out ICH in stroke patients before the administration of levemopamil. This may enable early administration of levemopamil therapy, which is required in view of the narrow therapeutic window for stroke. Hemorrhagic transformation of cerebral ischemic infarction seems to be a common phenomenon, but from the results of this study it may be assumed that there is a low risk of aggravating intracerebral bleeding in patients under therapy with levemopamil. Taking into consideration also the low cardiovascular activity of levemopamil, its cerebrovascular selectivity, its high blood-brain barrier permeability, its favorable receptor-binding profile, and its capability to attenuate the increase in hippocampal glutamate levels after cerebral ischemia, this drug appears to be a safe and promising candidate for clinical trials, eg, in stroke, brain trauma, or peritumoral edema.

In contrast, heparin significantly increased ICH in the rat. The dose of heparin we used was 55 IU·kg⁻¹·min⁻¹. Thrombus formation was prevented in the rabbit in the dose range of 10 to 100 U·kg⁻¹·min⁻¹. Clinically, bolus injections of 5000 IU heparin are frequently used in...
patients with acute ischemic stroke.\textsuperscript{27,28} Enhancement of intracranial bleeding by heparin in the rat model of ICH is in keeping with the concern of many neurologists about adverse effects of anticoagulant therapy in stroke patients.\textsuperscript{25} It must be noted, however, that we have chosen a comparably high dosage of heparin in our experimental study to prove that adverse therapeutic effects can be demonstrated in this model of ICH. Furthermore, the infusion with heparin and levemopamil were given in the present study at the time of intracranial bleeding to evaluate possible adverse effects in the most critical situation of hyperacute ICH. In the subacute management of patients the use of heparin may be included in the therapy of ischemic stroke or ICH to prevent thromboembolic complications.\textsuperscript{37}

MRI has become a valuable tool for differential diagnosis of ICH in the clinical routine.\textsuperscript{32,38-40} In the present study the extent of ICH was determined by high-field MRI, which is better suited than low-field MRI.\textsuperscript{38} It has been suggested that gradient-echo MRI may be more sensitive than spin-echo MRI in displaying ICH, but recent investigations indicate that gradient-echo MRI may overestimate the size of ICH.\textsuperscript{41} Furthermore, the difference in sensitivity is not as pronounced with high-field MRI.\textsuperscript{39,40} Therefore, we used conventional spin-echo imaging in our studies on ICH. High signal intensity on T\textsubscript{2}-weighted spin-echo images of ICH may not only result from blood components but also from edema in adjacent brain tissue. Since it was beyond the scope of the present study to investigate the effect of levemopamil on brain edema, we used T\textsubscript{2}-weighted MRI, thereby avoiding erroneous overestimation of the size of the hemorrhage.

The typical appearance of the lesion on the T\textsubscript{2}-weighted images was an isointense or slightly hyperintense region surrounded by a hypointense rim. Our observation of these two zones of lesion after 24 hours is in accordance with histological results that have been obtained by Rosenberg et al\textsuperscript{24} in the same animal model. Furthermore, this zonal appearance of the hemorrhagic lesions on our T\textsubscript{2}-weighted images of the rat corresponds to images of patients in the transitional period from type A to type B after 30 to 60 hours, according to the classification of Yamada et al.\textsuperscript{42} Increased signal intensities in the central portion of hematomas during this transitional period have been ascribed to transient methemoglobin formation. The diminished signal intensity in the rim of the ICH may result from conversion of methemoglobin to deoxyhemoglobin when diffusion of oxygen into the surrounding ischemic tissue takes place. The finding that our T\textsubscript{2}-weighted images of ICH after 24 hours in the rat closely resemble those that have been obtained in patients between 30 and 60 hours\textsuperscript{42} suggests that the evolution of ICH occurs in the rat on a compressed time scale. Similar conclusions have been drawn from behavioral and histological investigations in this animal model.\textsuperscript{24}

In conclusion, MRI is not only valuable in the clinical diagnosis of ICH but is equally well suited in preclinical research with a rat model of ICH. Our study extends the use of MRI and spectroscopy in pharmaceutical research from the demonstration of drug efficacy in various indications\textsuperscript{4,43-45} to the investigation of possible adverse drug effects in clinically relevant animal models.

References


Aggravation of intracranial bleeding or conversion of a nonhemorrhagic lesion into a hemorrhagic one is a concern with a number of therapies, such as anticoagulation, antiplatelet treatment, induced arterial hypertension, or anticoagulation with heparin and (alleged) cerebral vasodilation with levemopamil in a rat model of intracerebral hemorrhage. Two comments the authors assume, on the basis of literature data, that the model is at least sensitive to anticoagulation. Second, their doses of levemopamil would lead to cerebral vasodilation with some properties reminiscent of spontaneous intracerebral hemorrhage. Two comments the authors assume, on the basis of literature data, that the model is at least sensitive to anticoagulation. Second, their doses of levemopamil would lead to cerebral vasodilation with some properties reminiscent of spontaneous intracerebral hemorrhage.


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